IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of
Hofstraat et al.

Application Serial No.:
09/380,336

Filed: November 23, 1999

For: DIAGNOSTIC NEODYMIUM (III) YTTERBIUM (III) OR ERBIUM (III) ION-LIGAND COMPLEXES

Attorney Docket No.: DVME-1009US

Group Art Unit: 1641

Examiner: G. Gabel

#14 11/7/01

RULE 132 DECLARATION

I, Klemens Brunner , Ph. D., graduated at Vienna University and an expert in the area of photoluminescence am working in the Polymers & Organic Chemistry department of Philips Nat Labs, the Netherlands in the field of Luminescence Organic Materials. I have read the patent application Serial No. 09/380,336 "Diagnostic Neodymium (III), Ytterbium (III) or Erbium (III) ion-ligand complexes" by Hofstraat et al., and I am aware of the state-of-the-art in the research area concerned.

I have understood the matter described in the patent application and have studied the patent by Wieder et al. (US 5,830,769) and by Kardos et al. (US 6,159, 686), which have been cited in the Office Action of August 3, 2001.

None of these two cited patents are relevant for the patent application by Hofstraat.

Wieder et al. (US 5,830,769)

In Wieder et al. lanthanide complexes containing a lanthanide ion complexed by a chelate, which is used to bind the lanthanide and to bring about efficient excitation through an organic sensitizer molecule bound to the chelate. Contrary to the patent application of Hofstraat, Wieder et al. describe a lanthanide complex with a second organic "sensitizer", not directly coupled to the lanthanide ion. The second organic sensitizer molecule in the application by Wieder et al. can either act as quencher or as enhancer of the lanthanide luminescence. When the organic molecule is applied as quencher, it can accept energy from the luminescent lanthanide – at the same time the luminescence of the lanthanide is diminished. In this first application the long-wavelength organic molecules, like fluorescein, rhodamine and phycobiliproteins, can be applied. Their application and mode of use, however, is entirely different than in the patent application of Hofstraat, where they are used to sensitize (**enhance, not diminish**) the luminescence of the lanthanide.

When in the application of Wieder et al. the organic sensitizer is used to enhance the lanthanide luminescence, the organic sensitizer should fulfill the well-known energetic constraints of the lanthanide emitters. The organic sensitizers demonstrated are able to excite the green-emitting lanthanide ion Terbium and the red-emitting lanthanide ion Europium. In view of the pertaining energetic considerations this means that the organic sensitizers are excited in the ultraviolet region (which is below 400 nm!). This means that the rare earth metal chelate is excited in the ultraviolet, and that the second fluorophore emits in the visible part of the spectrum. It is exactly this limitation of the well-known and well-documented literature on the Europium and Terbium emitters that is solved by the invention of Hofstraat.

A second advantage of the approach of Hofstraat is that the sensitizer is directly connected to the lanthanide ion. The energy transfer is extremely distance dependent. In the construction of Hofstraat the distance is optimized for transfer. In the application of Wieder et al. the sensitizer is added to the solution, so is present next to the lanthanide complex.

Kardos et al. (US 6,159, 686)

Kardos et al. teach inorganic lanthanide containing particles, which can be excited in the near-infrared part of the spectrum at high intensity and, after absorption by two or more photons emit in the visible part of the spectrum after upconversion. The excitation therefore is concerned with a multiphoton process. In a multiphoton process in general the existence of a "virtual" level is postulated, which is not a "real", existing, level of the molecule, but an imaginary level, which acts as an intermediate state in the excitation process. The two-photon process is under normal excitation conditions extremely inefficient. Only when very intense, very short light pulses are applied with extremely high peak intensities, some two-photon luminescence is generated. Mention is made in the patent of the presence of green emitting fluorescein in some assays; the fluorescein, however, is used for reference and does **not** play any role whatsoever in the sensitized excitation of the lanthanide ions.

The patent by Hofstraat describes an approach in which the **emission** of the selected lanthanide occurs in the **near-infrared** region. This emission can be generated by direct (single photon) excitation of the organic sensitizer in the visible part of the spectrum (at wavelengths longer than 400 nm). In contrast to the patent by Kardos et al. the patent by Hofstraat describes the use of sensitizers, which can be excited in the visible part of the spectrum with a single photon; Therefore in the latter case the normal requirements for (single photon) excitation apply.

The discussion on the detection system and radiation requirements taught by Kardos, in relation to the assay mixture described by Wieder et al. is irrelevant for the patent by Hofstraat for the simple reason that the mixture, comprising the lanthanides Europium and Terbium, as suggested by Wieder et al., cannot be excited at wavelengths above 400 nm, because of the energy considerations described above. For measurement of the assay mixtures described by Hofstraat much simpler excitation can be used than for the system of Kardos, which requires high intensity lasers for excitation. Laser excitation as is realistically necessary because of the two-photon nature of the excitation process in the patent of Kardos. For excitation of the assay mixture by Hofstraat also low power diode or lamp excitation can be applied. The same argument is valid for the assay described by Wieder et al. The assay follows a single photon excitation process, which can also be excited by low-power (non-laser) excitation sources. The difference between Hofstraat and Wieder et al. is that the assay of Hofstraat can be excited at wavelengths longer than 400 nm and the visible emitting lanthanides (Europium and Terbium), as used by Wieder et al. not. The invention of lanthanide complexes, which can be excited at longer wavelengths and which emit in the near-IR forms the basis for the application of Hofstraat. Particularly the use of longer wavelengths in medical assays is very useful, because short wavelength irradiation causes damage to the biomolecules and the tissue. Moreover, the short wavelength light is absorbed by the tissue so that the penetration of the light is limited. The assay of Hofstraat brings a solution to these problems.

Conclusion

In conclusion, the patent by Hofstraat describes original matter, which is not at all disclosed nor suggested in any one of Wieder and Kardos, alone or in combination.

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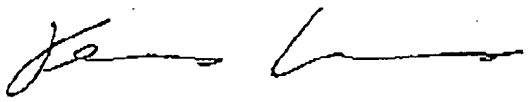
I declare that all statements made herein that are of my own knowledge are true and that all statements that are made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed: 

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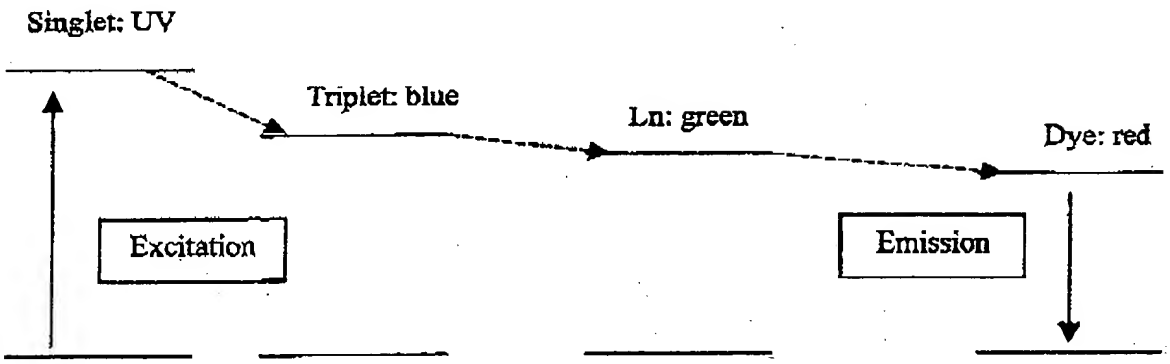
Diagrams describing the three (different) situations

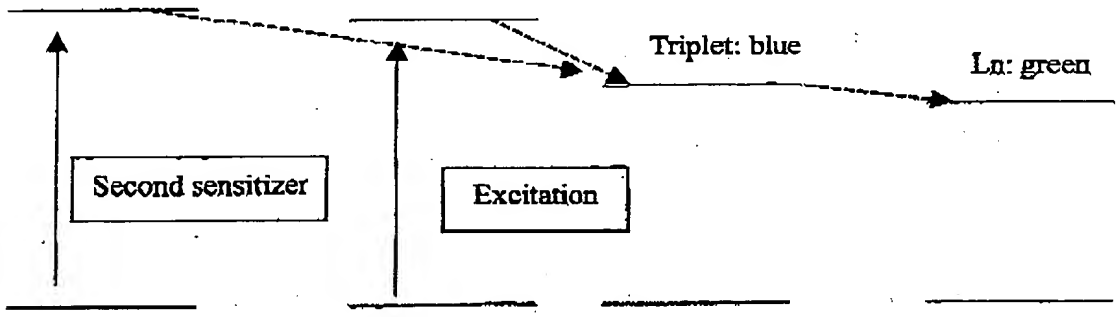
Figure 1: Wieder et al.

Case 1: Quenching of lanthanide luminescence



Sensitizer:	Lanthanide ion:	Acceptor (dye):
Singlet UV (<400 nm) excitation Energy transfer to triplet state	Transfer from triplet state of sensitizer to Tb excited state *Green* emission	Energy transfer from Tb, red emission, lanthanide emission is quenched

Case 2: enhancement of lanthanide emission



Enhancement through second sensitizer: energy should be sufficient to populate lanthanide excited state	Lanthanide sensitization further conventional

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Figure 2: Kardos et al.

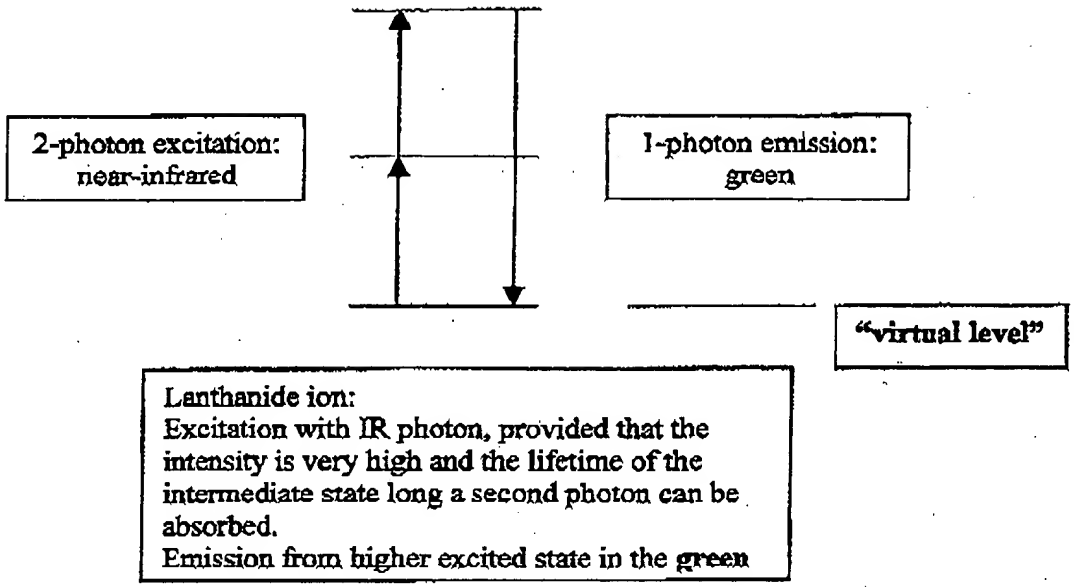

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Figure 3: Hofstraat